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Antimicrobial Drug Resistance Trends of Bacteremia Isolates in a Rural Hospital in Southern Mozambique

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Abstract. Antibiotic resistance in Africa is increasing but insufficiently recognized as a public health problem. However, there are scarce data for antimicrobial resistance trends among bloodstream isolates in sub-Saharan Africa. Antimicrobial drug resistance trends among bacteria isolated from blood of children < 15 years of age admitted to the Manhiça District Hospital in Mozambique during May 2001–April 2006 were monitored by disk diffusion. We documented a linear trend of increasing resistance throughout the study period to chloramphenicol among isolates of Non-typhi *Salmonella* ($P < 0.001$), *Escherichia coli* ($P = 0.002$), *Staphylococcus aureus* ($P < 0.001$), and *Haemophilus influenzae* ($P < 0.001$). Increasing resistance to ampicillin was also observed for *H. influenzae* isolates ($P < 0.001$). We report trends of increasing resistance among the most frequent etiologies of bacteremia to the most commonly used antibiotics for empirical therapy in this community. Quinolones and third-generation cephalosporines may be needed in the short term to manage community-acquired infections.

INTRODUCTION

Developing countries, especially in Africa, have a disproportionate burden of global childhood mortality caused by infectious diseases.[1] Invasive bacterial infections are major contributors to this excess mortality among children.[2,3] Non-typhi *Salmonella* (NTS), *Streptococcus pneumoniae*, *Staphylococcus aureus, Escherichia coli*, and *Haemophilus influenzae* have been consistently described as the principal bacteremia etiologies among children in sub-Saharan Africa.[2–6]

There is a growing concern regarding management of community-acquired infections in Africa because of increasing prevalence of resistance to the most commonly antibiotics used in these settings, and the emergence of multidrug-resistant strains.[6–11] Factors such as indiscriminate use of antibiotics as growth promoters in veterinary medicine, antibiotic dispensation without prescription, or incomplete compliance to prescribed duration of treatment are among the major contributors for the global increase of resistance. Intrinsic factors related to the appearance of antimicrobial drug resistance also take into account diverse molecular mechanisms of resistance, including the presence of plasmids or integrons carrying genetic determinants of resistance.[12–15] However, data for antimicrobial resistance, especially regarding trends, remain scarce throughout sub-Saharan African settings.[8–10]

In Mozambique, microbiology facilities are scarce and as a consequence, antibiotic therapy is mostly empirical. Empirical treatment in inpatients in Manhiça District Hospital follows Mozambican National guidelines and includes parenteral chloramphenicol or the combination of penicillin plus chloramphenicol for children greater than two months of age, or the combination of ampicillin plus gentamicin among younger children (< 2 months of age) or severely malnourished children. When *in vitro* susceptibility data are available, empirical treatment is re-assessed and changed when necessary, and ceftriaxone, when available, is used in cases of multidrug-resistance. However, there little data are available to monitor antibiotic resistance[16–19] and support national treatment guidelines. Within a large prospective study designed to characterize the etiology of bacteremia in children admitted to a rural district hospital,[3] we have been able to monitor antibiotic resistance over a five-year period. We analyzed the antimicrobial susceptibility patterns of bloodstream isolates and their time trends for the five most frequent etiologies of bacteremia found in this setting.

MATERIALS AND METHODS

Study site and population. The study was conducted at the Manhiça District Hospital, a 110-bed referral health facility for Manhiça District, a rural area located 80 km north of Maputo Province in southern Mozambique. The climate of the area is sub-tropical with two distinct seasons: a warm and rainy season from November through April and a cool and dry season during the rest of the year. The district has an estimated population of 140,000 inhabitants. The Manhiça district is located at 25°24′S, 32°48′E and has an average altitude of 50 meters above mean sea level. A full description of the geographic and socio-demographic characteristics of the study community has been reported elsewhere.[20]

Laboratory procedures. Antimicrobial susceptibility of bacteremia isolates from children less than 15 years of age admitted to the Manhiça District Hospital during May 2001–April 2006 were analyzed. Blood cultures were performed upon admission for all children less than two years of age and for children 2–14 years of age with axillary temperatures ≥ 39°C or with any sign of clinical severity and processed for bacterial isolation as detailed elsewhere.[3]

Antimicrobial susceptibility testing was performed by standard disk diffusion methods to trimethoprim/sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), oxacillin (1 μg), penicillin (10 μg), ampicillin (10 μg), gentamicin (10 μg), and erythromycin (15 μg) (Mast Group, Ltd., Merseyside, United

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Kingdom). Interpretation of resistance categories for isolates was done according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.[21] For oxacillin-resistant *S. pneumoniae* strains, penicillin minimum inhibitory concentrations were determined using E-test strips (AB Biodisk, Solna, Sweden). The NTS and *E. coli* isolates were also tested for resistance to nalidixic acid (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), and amoxicillin/clavulanic acid (20/10 μg). Additionally, phenotypic methicillin-resistant *S. aureus* (MRSA) were determined by oxacillin disk and cefoxitin disk and tested for resistance to vancomycin. *Escherichia coli* (ATCC 25922) and *S. aureus* (ATCC 25923) strains were used as internal quality control for assessing the adequacy of antibiotic disks.

Definitions. Multidrug resistance was defined as complete resistance to two or more unrelated antimicrobial agents. We considered non-susceptible isolates those with intermediate or full resistance.

Statistical analysis. Data were double-entered into a FoxPro database by using visual FoxPro version 2.6 (Microsoft Corp., Redmond, WA). The two entries were compared and discrepancies were resolved by referring to the original forms. Statistical analysis was performed by using STATA software version 9.0 (Stata Corp., College Station, TX). Proportions were compared using the chi-square test or Fisher's exact test as appropriate. P values < 0.05 were considered significant. Antimicrobial resistance trends for the most frequent etiology of bacteremia over the years of surveillance were analyzed by using the chi-square test for trend.

RESULTS

Antimicrobial drug susceptibility. Over the five-year study period, 19,896 blood cultures were collected from 23,686 admitted children less than 15 years of age and bloodstream episodes confirmed in 8%.[3] These isolates were tested for antibiotic susceptibility. The proportion of non-susceptible isolates to the antibiotics commonly used in this setting is shown in Table 1.

The NTS isolates showed a high rate of non-susceptible isolates to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, but only 3% (8 of 307) of the isolates were non-susceptible to nalidixic acid and all were susceptible to ciprofloxacin (0 of 307) or ceftriaxone (0 of 48). *Salmonella* Typhi, an uncommon bacteria in Manhiça (only threes isolates), was in all cases susceptible to all antibiotics tested. Eleven percent (17 of 148) of *E. coli* isolates showed resistance to nalidixic acid and despite the few isolates tested, no resistance was observed to ceftriaxone (0 of 18). Eight percent (14 of 176) of *S. aureus* isolates were fully resistant to oxacillin and one isolate (1%, 1 of 176) showed intermediate resistance. However, all of these isolates were susceptible to vancomycin. *Streptococcus pneumoniae* isolates were highly susceptible to penicillin and chloramphenicol, but a high prevalence of resistance was found to trimethoprim/sulfamethoxazole. Resistance among *H. influenzae* was high (≥ 46%, 51 of 111) for ampicillin, chloramphenicol, or trimethoprim/sulfamethoxazole.

Multidrug resistance and antibiotic combinations. Multidrug resistance was observed among 92% (142 of 155) of *E. coli* isolates, 67% (265 of 396) of NTS, 50% (56 of 111) of *H. influenzae,* 43% (81 of 188) of *S. aureus*, and 5% (20 of 386) of *S. pneumoniae*. Resistance to the most commonly used combination of ampicillin/gentamicin was high among *E. coli* isolates (29%, 42 of 147) and NTS (16%, 61 of 387). Furthermore, 20% (30 of 148) of *E. coli* isolates showed intermediate resistance to amoxicillin/clavulanic acid. Resistance to amoxicillin/clavulanic acid was 38% (113 of 297), with almost 20% of NTS isolates showing intermediate resistance.

We also evaluated antimicrobial resistance to chloramphenicol and different combinations of antibiotics according to the clinical syndromes (pneumonia, meningitis, acute gastroenteritis) that define, when present, empirical treatment upon admission (Table 2). A high frequency of non-susceptibility to chloramphenicol among the different clinical diagnoses was found, ranging from 32% (for meningitis) to 51% (for acute gastroenteritis). The level of resistance to ampicillin/gentamicin was approximately 16% for the syndromes analyzed, and the proportion of non-susceptibility to the combination of penicillin/gentamicin was 7% for meningitis diagnoses.

Trends of antimicrobial drug resistance. Trends of antimicrobial resistance over the five years of surveillance for the five most frequent bacteremia-causing isolates were evaluated. The trends of resistance to chloramphenicol, trimethoprim/sulfamethoxazole, and ampicillin for the five most frequent etiologies of bacteremia are shown in Figures 1 and 2. For chloramphenicol, a linear trend of increasing resistance was

TABLE 1

Frequencies of non-susceptible isolates to the most commonly used antibiotics, Mozambique*

Isolate	Ampicillin, n/N (%)	Chloramphenicol, n/N (%)	Cotrimoxazole, n/N (%)	Gentamicin, n/N (%)	Erythromycin, n/N (%)	Penicillin, n/N (%)
NTS	294/395 (74)	215/393 (55)	257/390 (66)	62/387 (16)	NA	NA
Streptococcus pneumoniae	37/326 (11)	28/384 (7)	158/360 (44)	NA	7/376 (2)	37/326 (11)
Staphylococcus aureus	163/182 (90)	69/186 (37)	55/177 (31)	10/182 (5)	63/180 (35)	163/182 (90)
Escherichia coli	148/154 (96)	121/155 (78)	127/141 (90)	42/148 (28)	NA	NA
Haemophilus influenzae	51/111 (46)	55/109 (50)	77/100 (77)	12/107 (11)	NA	NA
S. pyogenes	0/38 (0)	2/37 (5)	6/36 (17)	18/35 (51)	2/35 (6)	0/38 (0)
Group D *Streptococcus*	2/35 (6)	20/36 (56)	12/34 (35)	30/36 (83)	22/36 (61)	3/33 (9)
S. agalactiae	0/35 (0)	10/35 (29)	5/34 (15)	33/33 (100)	11/34 (32)	0/35 (0)
NF GNB oxidase positive	25/33 (76)	21/33 (64)	23/33 (70)	8/32 (25)	NA	NA
NF GNB oxidase negative	18/32 (56)	16/31 (52)	27/32 (84)	5/31 (16)	NA	NA
Enterobacter spp.	18/20 (90)	7/20 (35)	4/20 (20)	2/18 (11)	NA	NA
Neisseria spp.	0/9 (0)	0/12 (0)	3/11 (27)	0/9 (0)	NA	0/12 (0)
Other *Enterobacteria*	7/13 (54)	11/13 (85)	7/12 (58)	0/12 (0)	NA	NA
Klebsiella spp.	12/12 (100)	6/11 (55)	7/10 (70)	2/11 (18)	NA	NA
Pseudomonas spp.	8/10 (80)	6/10 (60)	7/10 (70)	0/10 (0)	NA	NA

*n = number of isolates non-susceptible (intermediate or full resistance); N = total number of isolates tested; NTS = Non-typhi *Salmonella*; NA = not applicable; NF GNB = non-fermenting Gram-negative bacilli.

TABLE 2

Frequencies of non-susceptibility to chloramphenicol and combinations of antibiotics according to gram stain and four common clinical syndromes, Mozambique*

Clinical diagnosis	Chloramphenicol, n/N (%)	Ampicillin plus gentamicin, n/N (%)	Penicillin plus gentamicin, n/N (%)	Chloramphenicol plus ampicillin, n/N (%)
Pneumonia	185/529 (35)	70/512 (14)	28/253 (11)	150/511 (29)
Meningitis	15/47 (32)	7/43 (16)	2/28 (7)	11/43 (26)
Acute gastroenteritis	80/161 (51)	22/158 (14)	–	74/157 (47)

*n = number of isolates non-susceptible (intermediate or full resistance); N = total number of isolates tested.

found among NTS (from 26% to 63%; $P < 0.001$), *S. aureus* (from 16% to 35%; $P < 0.001$), *E. coli* (from 62% to 92%; $P = 0.002$), and *H. influenzae* (from 10% to 94%; $P < 0.001$). For trimethoprim/sulfamethoxazole, a linear trend was also observed among *S. pneumoniae* (from 33% to 54%, $P < 0.001$), *S. aureus* (from 22% to 42%, $P = 0.002$), and *H. influenzae* (from 57% to 95%; $P = 0.005$). For ampicillin, an increase in resistance was high (from 19% to 75%; $P < 0.001$) among *H. influenzae*.

DISCUSSION

This report presents the first set of data regarding current levels and time trends of antibiotic resistance of the most commonly isolated bacteria from children admitted to a rural hospital in Mozambique. Data generated in this study show that the most available and inexpensive antibiotics commonly used in the area as empirical therapies for invasive bacterial infections have limited *in vitro* activity against the most frequent etiologies of bacteremia. Although tested in a small number of isolates, quinolones and ceftriaxone remain effective. Therefore, on the basis of our data, oral ciprofloxacin, now within reasonable costs, could constitute a good alternative for treatment of patients with invasive bacterial infections. However, resistance should be monitored because nalidixic acid–resistant *E. coli* strains were found in the present study and may predict future resistance to ciprofloxacin.[22–24] Although fluoroquinolones have recently been demonstrated to be safe in children,[25] they are rarely used in Mozambique. Use of third-generation cephalosporins, which appear to be an alternative for severe invasive disease and in children with infected with human immunodeficiency virus (HIV) or malnutrition in sub-Saharan Africa[2,4,26] is limited by its high cost in countries such as Mozambique.

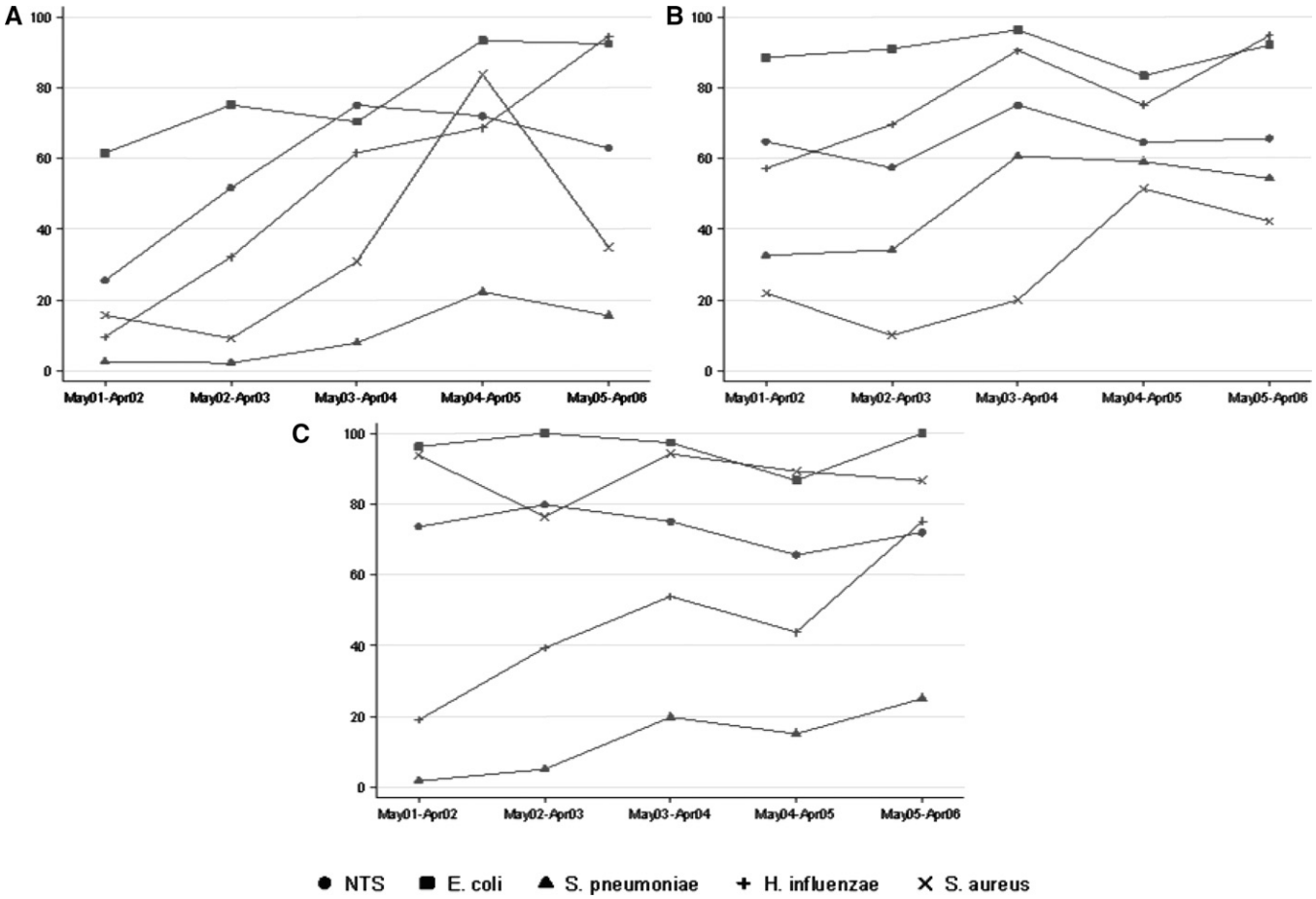

FIGURE 1. Proportion of non-susceptible isolates of the five most frequent agents of bacteremia over a five-year period (May 2001–April 2006) to **A**, chloramphenicol, **B**, trimethoprim-sulfamethoxazole, and **C**, ampicillin, Mozambique.

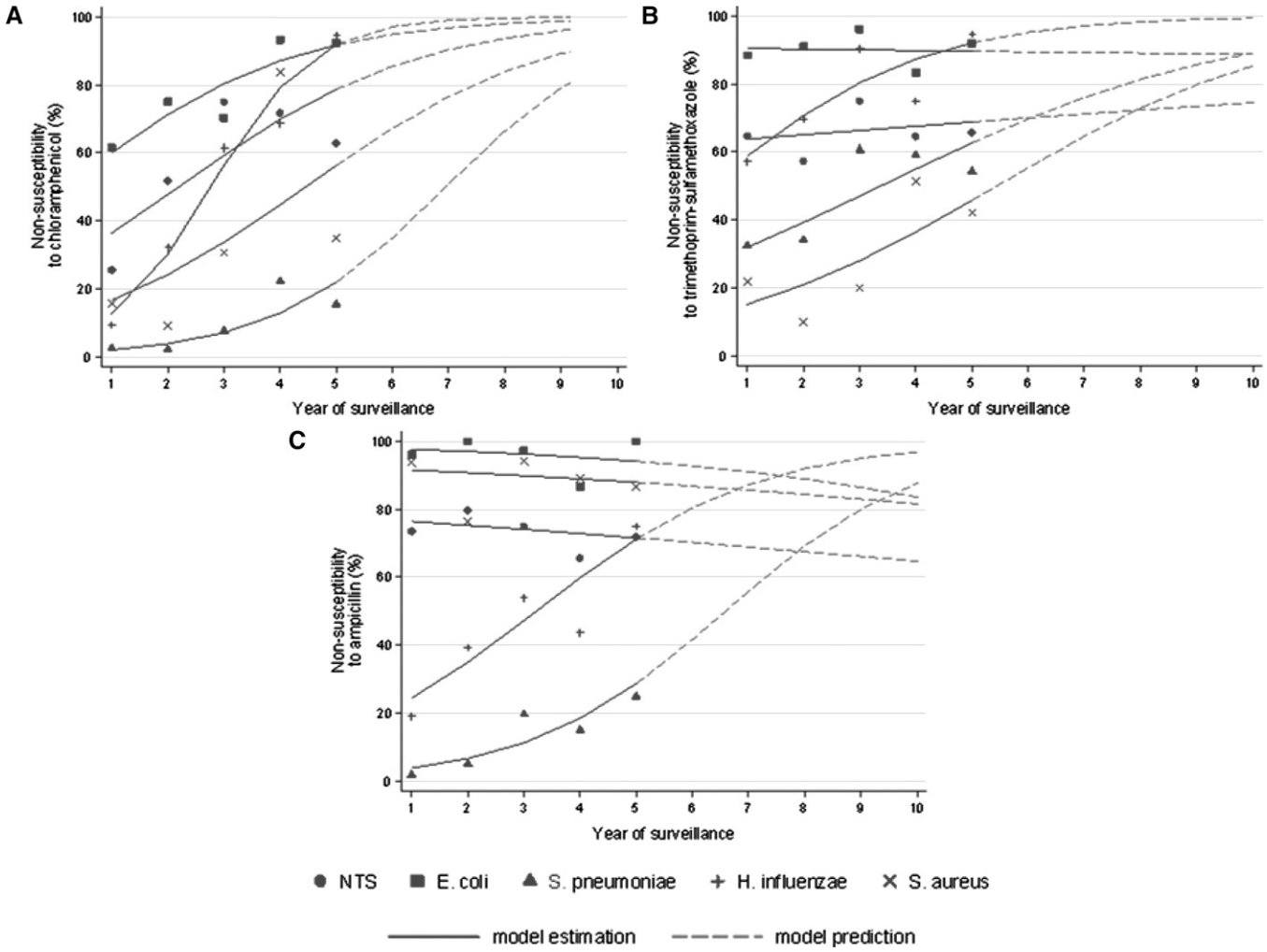

FIGURE 2. Estimated and predicted proportions of non-susceptibility of the five most frequent agents of bacteremia over a five-year period to **A**, chloramphenicol, **B**, trimethoprim-sulfamethoxazole, and **C**, ampicillin, Mozambique.

Factors such as incomplete compliance with antibiotic therapy, which is a well-known determinant of resistance in developing countries[14,15] and is thought to be common in the area; molecular mechanisms of resistance or the presence of genetic elements such as plasmids or integrons;[12,13] and migration, particularly to South Africa, which is common in this community,[27] may contribute to global high global level of resistance found in this study.

Rates of resistance for specific pathogens reported here are comparable to rates in other studies in sub-Saharan Africa.[6,9,10,13] The proportion of *S. aureus* isolates resistant to oxacillin is of concern because vancomycin is not available in the study area. The proportion of MRSA in this study was almost double than that previously reported in the country,[18] but lower than those reported from South Africa.[28,29] MRSA isolates usually arise from nosocomial infections, and often are associated with high mortality,[30] but community-acquired MRSA has also been reported elsewhere.[18,31] However, the origin of MRSA in our study is not clear because 5 of the 14 children with MRSA were from the demographic surveillance area, and records obtained through the morbidity surveillance system confirm that they had been admitted to the hospital at least once in the preceding four weeks, which suggests that these MRSA may be nosocomial.

One of the study limitations is that we have been unable to quantify the precise number of patients treated with inadequate antibiotics and their associated outcome because of lack of complete information on the number of doses of antibiotics given to a child and changes in antibiotics.

We also demonstrated trends of increasing resistance for the most frequent etiologies of bacteremia for chloramphenicol, which may be explained by its widespread use as empirical treatment among admitted children, leading to a high antibiotic pressure. The trend of resistance found for trimethoprim/sulfamethoxazole may be a legacy of its indiscriminate use in the past and also linked to common use for malaria treatment of sulfadoxine-pyrimetamine, a related antimicrobial drug that has been demonstrated to show produce cross-resistance. Furthermore, the prevalent mechanisms of trimethoprim resistance are located in the same genetic structures conferring resistance to other antibiotics used in the area.[32] Currently, trimethoprim/sulfamethoxazole is reserved for HIV-related opportunistic infection prophylaxis among HIV-infected patients. Our study is one of the few studies that have demonstrated the trend of antimicrobial resistance to the most commonly used and inexpensive antibiotics in developing countries for the most frequent etiologies of bacteremia in sub-Saharan Africa.[8,10]

In summary, our study suggests a trend of increasing antimicrobial drug resistance for the most commonly used antibiotics for empirical therapy, and quinolones and third-generation cephalosporins may be needed in the short term to manage community-acquired infections at the study site. Although the re-assessment of current national guidelines for antibiotic use is now crucial, bacterial surveillance systems should be implemented in other areas of the country to provide data on the etiology and prevailing antimicrobial drug resistance patterns of community-acquired agents causing bacteremia.

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REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE, 2005. WHO estimates of the causes of death in children. *Lancet 365:* 1147–1152.
2. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, Ngetsa C, Slack MP, Njenga S, Hart CA, Maitland K, English M, Marsh K, Scott JA, 2005. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med 352:* 39–47.
3. Sigauque B, Roca A, Mandomando I, Morais L, Quinto L, Sacarlal J, Macete E, Nhamposa T, Machevo S, Aide P, Bassat Q, Bardaji A, Nhalungo D, Soriano-Gabarro M, Flannery B, Menendez C, Levine MM, Alonso PL, 2009. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J 28:* 108–113.
4. Bachou H, Tylleskar T, Kaddu-Mulindwa DH, Tumwine JK, 2006. Bacteraemia among severely malnourished children infected and uninfected with the human immunodeficiency virus-1 in Kampala, Uganda. *BMC Infect Dis 6:* 160.
5. Walsh AL, Phiri AJ, Graham SM, Molyneux EM, Molyneux ME, 2000. Bacteremia in febrile Malawian children: clinical and microbiologic features. *Pediatr Infect Dis J 19:* 312–318.
6. Bahwere P, Levy J, Hennart P, Donnen P, Lomoyo W, Dramaix-Wilmet M, Butzler JP, De Mol P, 2001. Community-acquired bacteremia among hospitalized children in rural central Africa. *Int J Infect Dis 5:* 180–188.
7. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, Msangi V, Tellevik MG, Holberg-Petersen M, Harthug S, Maselle SY, Langeland N, 2007. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infect Dis 7:* 43.
8. Kariuki S, Revathi G, Kariuki N, Muyodi J, Mwituria J, Munyalo A, Kagendo D, Murungi L, Anthony Hart C, 2005. Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994–2003. *Int J Antimicrob Agents 25:* 38–43.
9. Bejon P, Mwangi I, Ngetsa C, Mwarumba S, Berkley JA, Lowe BS, Maitland K, Marsh K, English M, Scott JA, 2005. Invasive Gram-negative bacilli are frequently resistant to standard antibiotics for children admitted to hospital in Kilifi, Kenya. *J Antimicrob Chemother 56:* 232–235.
10. Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, Zijlstra EE, Heyderman RS, Hart CA, Molyneux ME, 2008. Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *S. enterica* Serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis 46:* 963–969.
11. Scott JA, Mwarumba S, Ngetsa C, Njenga S, Lowe BS, Slack MP, Berkley JA, Mwangi I, Maitland K, English M, Marsh K, 2005. Progressive increase in antimicrobial resistance among invasive isolates of *Haemophilus influenzae* obtained from children admitted to a hospital in Kilifi, Kenya, from 1994 to 2002. *Antimicrob Agents Chemother 49:* 3021–3024.
12. Mandomando I, Jaintilal D, Pons MJ, Valles X, Espasa M, Mensa L, Sigauque B, Sanz S, Sacarlal J, Macete E, Abacassamo F, Alonso PL, Ruiz J, 2009. Antimicrobial susceptibility and mechanisms of resistance in *Shigella* and *Salmonella* isolates from children under five years of age with diarrhea in rural Mozambique. *Antimicrob Agents Chemother 53:* 2450–2454.
13. Ruiz J, Herrera-Leon S, Mandomando I, Macete E, Puyol L, Echeita A, Alonso, 2008. Detection of *Salmonella enterica* serotype Typhimurium DT104 in Mozambique. *Am J Trop Med Hyg 79:* 918–920.
14. Planta MB, 2007. The role of poverty in antimicrobial resistance. *J Am Board Fam Med 20:* 533–539.
15. Hart CA, Kariuki S, 1998. Antimicrobial resistance in developing countries. *BMJ 317:* 647–650.
16. Valles X, Flannery B, Roca A, Mandomando I, Sigauque B, Sanz S, Schuchat A, Levine M, Soriano-Gabarro M, Alonso P, 2006. Serotype distribution and antibiotic susceptibility of invasive and nasopharyngeal isolates of *Streptococcus pneumoniae* among children in rural Mozambique. *Trop Med Int Health 11:* 358–366.
17. Sigauque B, Roca A, Sanz S, Oliveiras I, Martinez M, Mandomando I, Valles X, Espasa M, Abacassamo F, Sacarlal J, Macete E, Nhacolo A, Aponte J, Levine MM, Alonso PL, 2008. Acute bacterial meningitis among children, in Manhica, a rural area in Southern Mozambique. *Acta Trop 105:* 21–27.
18. Ceccarelli D, Mondlane J, Sale M, Salvia AM, Folgosa E, Cappuccinelli P, Colombo MM, 2005. Sporadic methicillin resistance in community acquired *Staphylococcus aureus* in Mozambique. *New Microbiol 28:* 327–336.
19. Roca A, Quinto L, Abacassamo F, Morais L, Valles X, Espasa M, Sigauque B, Sacarlal J, Macete E, Nhacolo A, Mandomando I, Levine MM, Alonso PL, 2008. Invasive *Haemophilus influenzae* disease in children less than 5 years of age in Manhica, a rural area of southern Mozambique. *Trop Med Int Health 13:* 818–826.
20. Alonso PL, Saute F, Aponte JJ, Gómez-Olivé FX, Nhacolo A, Thompson R, Macete E, Abacassamo F, Ventura PJ, Bosch X, Menédez C, Dgedge M, 2002. *Manhiça DSS, Mozambique. Population and Health in Developing Countries*. Volume 1. Ottawa, Ontario, Canada: INDEPTH, 189–195.
21. Clinical and Laboratory Standard Institute (CLSI), 2006. *Performance Standards for Antimicrobial Disk Susceptibility Tests. Ninth Edition; M2-A9*. Wayne, PA: CLSI.
22. Ruiz J, 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother 51:* 1109–1117.
23. Ruiz J, Gomez J, Navia MM, Ribera A, Sierra JM, Marco F, Mensa J, Vila J, 2002. High prevalence of nalidixic acid resistant, ciprofloxacin susceptible phenotype among clinical isolates of *Escherichia coli* and other Enterobacteriaceae. *Diagn Microbiol Infect Dis 42:* 257–261.
24. Pieroni P, Goodfellow J, Reesor L, Louie M, Simor AE, 1997. Antimicrobial susceptibilities of blood culture isolates obtained before and after the introduction of ciprofloxacin. *J Antimicrob Chemother 39:* 419–422.
25. Murray TS, Baltimore R, 2007. Pediatric uses of fluoroquinolone antibiotics. *Pediatr Ann 36:* 336–343.
26. Graham SM, English M, 2009. Non-typhoidal salmonellae: a management challenge for children with community-acquired

invasive disease in tropical African countries. *Lancet 373:* 267–269.
27. Nhacolo A, Nhalungo D, Sacoor CN, Aponte JJ, Thompson R, Alonso PL, 2006. Levels and trends of demographic indices in southern rural Mozambique: evidence from demographic surveillance in Manhiça district. *BMC Public Health 6:* 291.
28. Cotton MF, Wasserman E, Smit J, Whitelaw A, Zar HJ, 2008. High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing *Enterobacteriaceae* and methicillin-resistant *Staphylococcus aureus* in nasopharyngeal and blood isolates of HIV-infected children from Cape Town, South Africa. *BMC Infect Dis 8:* 40.
29. Shitu AO, Lin J, 2006. Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu Natal Province, South Africa. *BMC Infect Dis 6:* 125.
30. Brink A, Moolman J, da Silva MC, Botha M, the National Antibiotic Surveillance Forum, 2007. Antimicrobial susceptibility profile of selected bacteraemic pathogens from private institutions in South Africa. *S Afr Med J 97:* 273–279.
31. Mulla S, Patel M, Shah L, Vaghela G, 2007. Study of antibiotic sensitivity pattern of methicillin-resistant *Staphylococcus aureus*. *Indian J Crit Care Med 11:* 99–101.
32. Dalsgaard A, Forslund A, Sandvang D, Arntzen L, Keddy K, 2001. *Vibrio cholerae* O1 outbreak isolates in Mozambique and South Africa in 1998 are multiple-drug resistant, contain the SXT element and the aadA2 gene located on class 1 integrons. *J Antimicrob Chemother 48:* 827–838.